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The effect of an intermittent water-table gradient on soil and xylem nitrate in cotton

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Summary Cotton (*Gossypium hirsutum* cv Deltapine 61) was grown in a sloping plot of soil in the field to examine the effect of a gradient of water-table depth on soil nitrate availability and plant uptake during two periods of the growing season. Before the water-table was imposed NO_3 was less concentrated at the lower end of the sloping plot. This was attributed to slow denitrification at microsites within the soil at the lower end which was wetter than further up the plot. At flooding NO_3 disappeared only slowly due to a carbon substrate limitation to denitrification in the soil. This loss occurred primarily in areas where the water-table was high and oxygen concentration in the soil solution was low. Plant NO_3 uptake, assessed by measuring the concentration in the xylem, parallelled the distribution of NO_3 in the soil solution. Under high water-tables xylem NO_3 levels fell but it was not possible to say whether this was due to impaired root function or to the reduced concentration of NO_3 observed in the soil solution. At intermediate water-table depths where soil NO_3 availability remained high xylem NO_3 concentration fell relative to the well drained control plants, suggesting that flooding had damaged the root system.

Introduction

Plants subjected to waterlogging often show nitrogen-deficiency symptoms[1]. However it is not always clear whether this is due to an anaerobiosis induced impairment of N uptake by roots[11] or the reduced availability of N caused by denitrification[3]. Nitrate is the major form of N taken up by cotton roots[5] and is also the N species most susceptible to loss from the soil during flooding. To examine the effect of varying degrees of inundation on soil NO_3 and plant NO_3 uptake a sloping plot was used to provide a gradient of water-table depth at two times in the growth of a cotton crop. The uptake and partitioning of N within the cotton plants of this study has been described previously[4].

Material and methods

Site and plants

Details of the construction of the sloping plot have been given elsewhere[7]. Its dimensions were 45 m long × 6 m wide × 0.6 m deep with a slope of 1.78%. When flooded this formed a water-table

*Manufactured by Merck. Mention of commerical names does not imply endorsement by either CSIRO or USDA.

that was 40 mm above the soil and 50, 170, 270, 380 and 660 mm below the surface at the six measuring positions 3, 9, 15, 21, 27 and 43 m respectively from the lower end of the facility. The soil was repacked Hanwood clay loam with 200 mm of topsoil and 400 mm of subsoil. Nitrogen (130 kg urea-N ha^{-1}) and phosphorus (100 kg P ha^{-1}) fertilizers were applied with a combine fertilizer spreader in bands 160 mm apart and 20 mm deep on 25 October 1982 and incorporated by discing. A second application of 75 g N ha^{-1} urea was broadcast by hand on 9 February 1983 across the whole plot and watered in by sprinkler irrigation. The plot was sprinkler irrigated and in addition two flooding events were imposed to establish a water-table between (1) 17–25 January 1983 and (2) 7–23 March 1983 by subirrigating through pipes 700 mm below the soil surface.

Cotton (*Gossypium hirsutum* cv Deltapine 61) seeds were planted on 26 October 1982 and thinned to a density of 17 to 19 plants per m^2 at the seedling stage.

Soil samples

Soil samples were taken to estimate mineral N on 25 October 1982 just prior to the application of fertilizer and immediately before (13 Jan 1983) and after (25 Jan 1983) Flood 1, and before (3 Mar 1983) and after (23 Mar 1983) Flood 2. For each sampling 3 replicate soil cores (45 mm dia.) were removed from the sloping plot to a depth of 600 mm. Each core was measured for length and then cut transversely into 4 equal depth increments, placed into aluminium cans, sealed and transported to the laboratory. Within 3 h the soil segments were crumbled, homogenised by hand and 20 g subsamples taken for mineral N extraction. The remainder was weighed and dried at 110°C for moisture determination. After shaking the 20 g subsamples for 30 min with 200 ml of water, 30 g of KCl was added and the shaking continued for 1 h. The suspension was allowed to settle (12–24 h) and 4 ml of the clear supernatant was taken for analysis of NO_3 (after reduction with hydrazine) and NO_2 by the N-(1-naphthyl)-ethylenediamine reaction.

Soil solution oxygen and nitrate

Water samples were taken from submerged probes at selected positions in the sloping plot. The probes were set at depths of 100, 200, 350 and 550 mm but it was only possible to withdraw aqueous samples from those that were either beneath or slightly above the water-table using glass syringes. The initial 20 ml was discarded to ensure that the sample solution for analysis came directly from the soil (probe void volume was approximately 13 ml). Samples were assayed for oxygen with an amperometric oxygen electrode. NO_3 was assayed by the previously mentioned reaction and by Merckoquant Nitrate test indicator strips*. In a few samples where NO_2 was detected the colour value for this was subtracted to give a better estimate of the true NO_3 concentration.

Soil NO_3 utilization test

Soil cores from the 3 m position at the lower end of the sloping plot were taken back to the laboratory after the first flooding event. 20 g subsamples of the moist soil from different depth segments were placed in 500 ml jars with 100 ml of 2 m*M* KNO_3. Glucose was added to half the samples to a final concentration of 1% w/v. 4 ml aliquots of solution were taken at 0, 2 and 7 days after the start of the incubation for NO_3 estimation. The incubation temperature was 25°C, and pH in the range 7.0–7.4.

Xylem sap NO_3

Xylem sap was expressed from the petiole of leaves enclosed in a Scholander pressure chamber used to measure leaf water potential[10]. This was directly absorbed onto a NO_3 test strip and an estimate of concentration made from the colour developed. This test provided an estimate of NO_3 in the petiolar xylem sap as distinct from that in the whole tissue[9].

Results and discussion

Soil nitrate

Although both NH_4 and NO_3 were measured, NH_4 was found to occur in only low concentrations (0.1–4.1 μg N cm^{-3}) and was therefore not

Table 1. Concentration of NO_3 in the soil (NO_3 $\mu g\,N\,cm^{-3}$). Each value from 3 replicate soil cores averaged above and below the water-table expressed on a per volume of soil basis. ($P < 0.05$, Mann Whitney test)

Position along plot	3 m		22 m		42 m	
Prefertilizer						
Above	—		35.5		20.9	
Below	11.1		12.1		—	
Flood 1	before	after	before	after	before	after
Above	—	—	30.4 (ns)	55.9	22.0 (ns)	22.6
Below	2.5 (ns)	7.9	32.8 (ns)	19.8	—	—
Flood 2						
Above	—	—	32.5 (ns)	16.0	26.3 (ns)	3.8
Below	1.4 (ns)	0.6	21.4 (ns)	18.6	—	—

considered further. NO_3 varied widely in concentration at any particular position within the sloping plot both before and after flooding, despite attempts to obtain a uniform distribution (Table 1). Similarly there was no consistent pattern of vertical distribution of NO_3 within the soil. Because of this variability it was impossible to detect significant changes in the soil NO_3 concentration either above or below the water-table during either flooding event. However NO_3 concentrations were consistently lower at the bottom end of the sloping plot. This was true either before fertilizer was applied or plants grown, as well as before and after the two flooding events.

The low NO_3 concentrations were possibly related to the increased moisture content of the soil at the lowest end of the plot (Table 2). Wetter soil resulted from both the observed surface run-off and expected subsurface movement of water through the soil during sprinkler irrigation. This might have favoured the loss of NO_3 through denitrification within small soil aggregates even though most of the soil was not saturated[2].

Despite the large variability, high NO_3 concentrations found in some soil cores below the water-table suggested that NO_3 utilisation was slow. Laboratory incubation of soil taken from the sloping plot confirmed this. Addition of carbon substrate (glucose) however, resulted in a rapid disappearance of NO_3, especially in the topsoil sample (Table 3). It was

Table 2. Volumetric water content of soil samples referred to in table 1 averaged to a depth of 600 mm

Position along plot	3 m	22 m	42 m
Prefertilizer	0.26 ± 0.02	0.26 ± 0.01	0.29 ± 0.01
Before flood 1	0.33 ± 0.02	0.25 ± 0.01	0.28 ± 0.01
After flood 1	0.38 ± 0.01	0.36 ± 0.01	0.32 ± 0.01
Before flood 2	0.33 ± 0.01	0.28 ± 0.01	0.25 ± 0.01
After flood 2	0.38 ± 0.01	0.36 ± 0.01	0.36 ± 0.01

Table 3. Effect of added glucose on NO_3 concentrations (m*M*) in flooded soil in the laboratory

Depth (mm)	Zero time		2 days		7 days	
	−*	+*	−*	+*	−*	+*
0–150	2.0	2.0	2.0	0.0	1.3	0.0
150–300	2.0	2.0	2.0	0.2	1.8	0.0
300–450	2.0	2.0	1.9	1.3	1.8	0.0
450–600	2.0	2.0	1.9	0.9	1.6	0.0

*Without (−) or with (+) glucose

likely therefore that the slow removal of NO_3 in the field was due to a limitation in the supply of suitable organic carbon even though a well developed cotton crop was present.

As an anion, NO_3 in the soil was likely to be in solution and not bound to the solid phase. Taking the soil solution from permanently installed probes ensured that samples were always withdrawn from the same zone within the plot and possibly helped to reduce some of the variability seen in the data from the soil cores. For some probes positioned beneath the water-table it was possible to withdraw gaseous samples suggesting that air had been trapped when the flooding was commenced. Both core and solution methods indicated the same overall pattern of NO_3 distribution, with a marked depression in concentration at the lower end of the plot (Fig. 1). In this area where the water-table was highest the already low NO_3 concentration fell to zero during the second flooding period. The first order rate constant for this loss at the lower end with high water-tables was found to be $2.3\,wk^{-1}$ which compared well with that for carbon limited flooded soil of Reddy *et al.*[6] ($2.4\,wk^{-1}$, Fig. 2). This was also an area where oxygen concentrations were low. In other places further up the sloping plot where there was better aeration NO_3 losses appeared to be smaller.

Xylem nitrate

A major sink for soil NO_3 is plant uptake. In cotton NO_3 taken up by the roots is largely passed to the leaves without undergoing reduction[5]. This upward flux of NO_3 can be represented as the product of the NO_3 concentration and transpiration rate. During the second flooding period transpiration was only slightly affected by high water tables[8] so that NO_3 uptake rates within the plot could be compared at any one time of day by considering the concentration of NO_3 in the xylem (Fig. 1). The distribution of xylem NO_3 in the xylem (Fig. 1). The distribution of xylem NO_3 along the sloping plot parallelled that seen for the soil solution, particularly at the start of the second flooding where these two were well correlated linearly ($r^2 = 0.90$). Soil solution NO_3 concentration clearly had a strong influence on the rate of NO_3 uptake by the plant

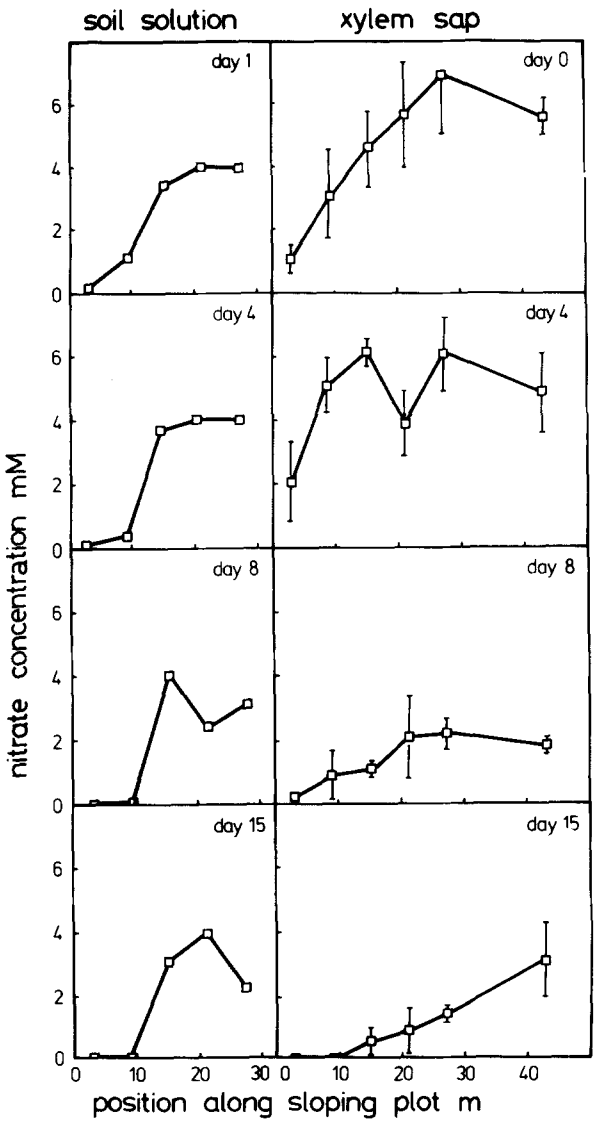

Fig. 1. Effect of flooding on soil solution and xylem NO_3 concentration during the second flooding event. Soil solution means are from 1 to 4 values and xylem sap means are from 4 samples. Day 0 is 7 March 1983.

and it was important to consider this factor when interpreting the plant uptake results.

Levels of NO_3 in the xylem fell during the second flooding even where the water table was low. On days 8 and 15 the weather was overcast and this could have reduced photosynthate dependent NO_3 uptake by the

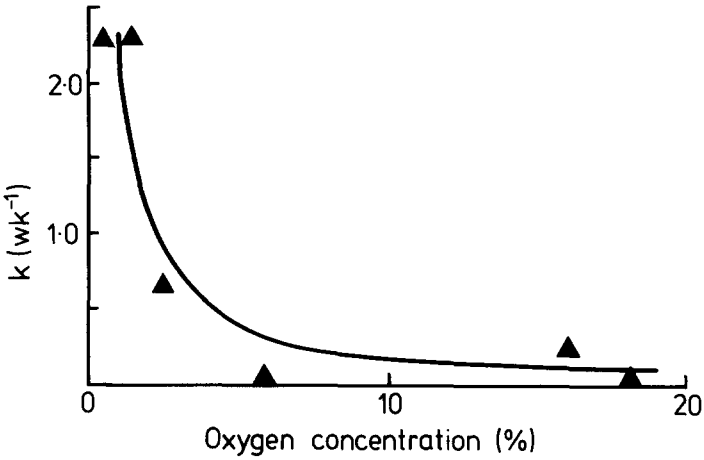

Fig. 2. Variation in the first order rate constant (k) for NO_3 disappearance from the soil solution as a function of oxygen concentration (air saturated = 21%) during the first 8 days of the second flooding event.

roots more severely than transpiration so that xylem concentrations fell. Whatever the explanation, such environmental effects, common to all positions within the plot could be removed by expressing the results relative to those from the lowest water table at the 42 m position. When this was done high water-table plants still contained much less NO_3 in their xylem than those with less of their root system submerged. However soil NO_3 availability was both low initially and declined in the lower part of the sloping plot (Fig. 1). This made it difficult to decide whether the high water-table at the 3 m position had not also affected the ability of the plants themselves to take up NO_3. However the disappearance of NO_3 from the soil solution seemed to occur only at low oxygen concentrations (Fig. 2) and this implied that anaerobic processes such as microbial denitrification were more likely to be involved than respiration dependent NO_3 uptake by roots. Further up the plot where NO_3 levels in the soil remained high, xylem concentrations only fell substantially (relative to the no water-table position at 42 m) after 8 days of flooding. Thus at these intermediate water-table depths there was evidence that submerging part of the root system had impaired its performance.

It was possible that the roots had adapted to high water-tables during the first flooding. Although there appeared to be little aerenchyma development in the stem or roots, a greater proportion of the roots was above the water-table[7]. Cotton, a tap-rooted plant, does not appear to develop as dense a root system as other crops (Meyer unpublished data) and this may have contributed to the incomplete depletion of soil oxygen

and low organic carbon supply. In addition the trapping of air beneath the water-table and the low metabolic activity of the soil (*e.g.* seen in the NO_3 utilisation tests, Table 3) might not have provided a sufficiently intense anaerobiosis to impair root function. It is conceivable therefore that a soil with more available organic carbon might have both denitrified faster and also inhibited root uptake functions to a greater extent.

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